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Preparation, antioxidant potential and angiotensin converting enzyme (ACE) inhibitory activity of gum arabic-stabilised magnesium orotate nanoparticles

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Abstract

The present work investigated the antioxidant properties and antihypertensive activity of magnesium orotate (MgOr) using various established *in vitro* assays, such as β-carotene bleaching activity, 1,1-diphenyl-2-picrylhydrazyl (DPPH), and nitric oxide scavenging activity as well as angiotensin converting enzyme (ACE) inhibitory activity. Magnesium orotate nanoparticles (MgOrGANPs) were prepared using the gum arabic (GA) as stabiliser coatings for nanoparticles through freeze-drying method. The *in vitro* cytoxicity of MgOrGANPs against human breast cancer MCF7, liver cancer HepG2, and colon cancer HT29 was investigated. The nitric oxide (NO) and DPPH scavenging assays of MgOrGANPs showed a dose-dependent trend, while 500 and 200 µL/mL were significantly more effective than the other concentrations with an IC_{50} of 89.56 µg/mL and 63.22% DPPH scavenging capacity respectively. The exposure of human cancer cells to MgOrGANPs at 1.56 – 1,000 µg/mL using 3-)4,5-dimethylthiazol-2-yl(2,5-diphenyl tetrazolium bromide (MTT) inhibited the growth of cell lines examined in a dose-dependent manner. Hence, MgOrGANPs may have great potential to be applied for cancer treatments.

Introduction

Various free radical scavengers of respiratory chain and prostaglandin synthesis are produced via the normal bodily physiological reactions during the process of respiration of aerobic organisms. The aforementioned biological consequences can be provoked by free radicals damaged deoxyribonucleic acid (DNA) bases but their excessive usage could result in damage and even kill cells. This, in turn, inflict various diseases like cancer and diabetes mellitus (Halliwell, 1994). Nevertheless, several studies have demonstrated that even the internal defence system is incapable of preventing human bodily reaction against reactive radicals, thereby requiring the need for alternative antioxidants (Kumar, 2011). In the past decades, there has been increased attention in evaluating the antioxidant activity of polymeric nanoparticles that are used as drug delivery systems (Hassani *et al.,* 2018). The emphasis is mainly related to antioxidant and the functioning of bio-systems due to the interactions between molecular oxygen and biomolecules in the bio-systems, which can produce harmful free radicals leading to metabolic perturbations (Das *et al.,* 2013). Therefore, antioxidant substances are mainly utilised to destroy toxic and harmful effects of these free radicals specifically by detoxifying organisms and scavenging the free radicals. Previous studies have confirmed the crucial role of food antioxidants especially those containing fats and oils to be protective against free radicals such as hydrogen peroxide and hydroxyl radical for the prevention of cancers and cardiovascular diseases (Kris-Etherton *et al.,* 2002; Serafini *et al.,* 2002). Besides, lipid peroxides generated from oxidised food have resulted in various diseases such as necrosis of epithelial tissues, stroke, and cancers. In this regard, synthetic antioxidants have been used for scavenging these radicals (Regoli *et al.,* 2000; Ryu *et al.,* 2012).

Various polymer-drug conjugates have been developed from gum arabic (GA) to enhance the antioxidant properties and therapeutic efficacy of drugs. GA is the exudate polysaccharide from gum acacia that possesses antioxidant properties and is widely used as a stabiliser or emulsifier for various pharmaceutical preparations (Anderson and Eastwood, 1989; Cadenas and Davies, 2000). Angiotensin-converting-enzyme inhibitors is an enzyme-related carboxypeptidase (ACE2) or dipeptidyl

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carboxypeptidase (EC 3.4.15.1), which could ameliorate patients from suffering a heart failure (Vickers *et al.,* 2002; Li *et al.,* 2004; Sarmadi *et al.,* 2011). Similarly, GA, which is a plant-based hydrocolloid, has also been reported to possess antioxidant and antihypertensive properties. Additionally, GA is also a well-known non-toxic polysaccharide with a stabilising effect and gelling characteristics (Abd-Allah *et al.,* 2002; Ali *et al.,* 2009).

Magnesium orotate (MgOr) is a complex of two synergic protective components: magnesium and orotic acid. Previous study has established the antihypertensive properties of MgOr (Hacht and Taaya, 2006). The challenge to retain the MgOr applications as anticancer and antioxidant has been resolved through the use of a natural polysaccharide, such as GA (Kostova *et al.,* 2007; Kostova and Valcheva-Traykova, 2015). As a highly heterogeneous compound, GA is incorporated with about 2% of polypeptide, which is an active substance that improves the antihypertensive effect. Furthermore, GA could enhance the stability and solubility of MgOr. In a previous study, GA demonstrated cytotoxicity effect against HepG2 cells due to the chemical binding of galactose groups with asialoglycoprotein receptor (ASGRP) on the hepatocytes for liver cancer delivery (Dodin and Dubois, 1980; Shon *et al.,* 2003; Kostova *et al.,* 2007).

Therefore, the present work aimed to develop a novel nanoparticle (NP) system using GA in order to improve the antioxidant and antihypertensive properties as well as the therapeutic efficacy of MgOr as an active ingredient. The antioxidant and antihypertensive properties of MgOr nanoparticles coated with GA (MgOrGANPs) were also assessed.

Materials and methods

Materials

Gum arabic polysaccharide was purchased from ENNASR (Sudan). RAW 264.7 cells were purchased from ATCC (American Type Culture Collection). Magnesium orotate was purchased from Richest (China). Angiotensin-converting enzyme, Trolox, hippuryl-histidyl-leucine sulphonamide, N-(1-naphtyl) ethylenediamine dihydrochloride, sodium nitrite, linoleic acid, dexamethasone, and Dulbecco's modified Eagle's medium (DMEM) high glucose were purchased from Sigma-Aldrich (USA). Tween 80, quercetin, *β*-carotene, and α-tocopherol were purchased from R&M (China). The entire work utilised deionised water.

Preparation of magnesium orotate nanoparticles

The MgOrGANPs were prepared using the freeze-drying method with slight modifications (Ashwaq *et al.,* 2017). Briefly, 0.94 g of MgOr was dissolved in 50 mL of deionised water in a 100 mL beaker and heated at 35°C under mild agitation for 20 min at room temperature. Then, 0.75 g of GA was dissolved in 50 mL of deionised water. In order to prepare an aqueous solution of MgOr and GA, MgOr solution was added dropwise to the GA solution with vigorous stirring for 72 h. The obtained mixture was mixed using Ultra-thorax at room temperature. This pre-emulsion was further passed through a ten cycles high pressure homogeniser at a pressure of 1,000 bar for 12 cycles to obtain a uniform mixture. The resulting clear solution was frozen at -80°C and then subsequently freeze-dried for 24 h at -55°C. The prepared powder having uniform properties was then used for characterisation.

Determination of antioxidant activity and cell viability

DPPH scavenging activity of MgOrGANPs

The DPPH scavenging assay is based on the change of purple DPPH radicals into yellow stable compound. This reaction has been related to the donating ability of the antioxidant (Barros *et al.,* 2007b). DPPH was prepared as a control solution by using 200 µL DPPH in 200 µM methanol then mixed with 100 µL of each sample solution (the samples were dissolved first in water) on a 96-well microplate and placed into a container protected from light. The mixture was homogenised by vortexing for 1 min before incubating for 1 h at room temperature in the dark. The absorbance rate of each solution was measured using a UV-Vis spectrophotometer at 517 nm. Trolox served as a positive control, while MgOr and MgOrGANPs were the test materials.

The stock solution of Trolox (100 µg/mL methanol) was prepared, and serially diluted to obtain a standard concentrations of 50, 75, 100, 125, 150, and 200 µg/mL. The percentage inhibition of the samples was calculated using Eq. 1:

$$\text{DPPH scavenging activity (\%)} = [1 - \frac{A_a - A_b}{A_c}] \times 100 \quad \text{(Eq. 1)}$$

Where, A_a, A_b, and A_c = absorbance of sample added into the DPPH solution, absorbance recorded of sample without DPPH, and the absorbance of the control, respectively. The IC_{50} value is the amount of antioxidants (MgOr and MgOrGANPs) needed to halve the initial DPPH concentration.

Nitric-oxide radical scavenging activity

The murine monocytic macrophage cell line RAW 264.7 were purchased from ATCC (USA), and cultured in DMEM including 100 units/mL penicillin, 100 µg/mL streptomycin, 2 mM glutamine, and 10% foetal bovine serum (FBS); then incubated at 37°C and 5% CO^2 for 72 h.

The RAW 264.7 cells were cultured in 96-well tissue cultures plates (1×10^6 cells/100 mL) at 37°C with 5% CO_2 for 24 h. After reaching 90% confluence, the RAW 264.7 cells were removed, trypsinised, and centrifuged at 120 *g* and 4°C for 10 min. Moreover, a serial dilution of MgOr and MgOrGANPs (1,000 µg/mL) in medium was prepared and added to the well to obtain final concentrations of 15.6, 31.25, 62.5, 125, 250, and 500 µg/mL. The cells were then challenged with 10 µg/mL of LPS for 20 h. Nitric oxide (NO) production was quantified by the measure of the quantity of nitrite released into the supernatant of macrophage cells. The Griess reagent [1% (w/v) sulphonamide and 0.1% (w/v) N-(1-naphtyl) ethylenediamine dihydrochloride in 2.5% (v/v) phosphoric acid] was used to quantify the amounts of nitrite oxidised with NO in the tissue culture media. To summarise, 100 µL of cell culture fluid was mixed with 100 µL of Griess reagent into in a 96-well tissue culture plate. The absorbance was then read spectrophotometrically using a microplate reader at 540 nm. Various concentrations of sodium nitrite were used to prepare a standard curve for the determination of nitrite concentration in the sample (Al-Qubaisi *et al.,* 2013; Ranneh *et al.,* 2016).

β-carotene bleaching assay (BCRA)

The principle of this assay is focused on the oxidation of linoleic acid, which leads to a decrease of the yellow colour of β-carotene. The linoleate model system was used to evaluate the antioxidant activities of MgOr and MgOrGANPs. Briefly, 2 mg of β-carotene was dissolved into 10 mL of chloroform to prepare the β-carotene solution. The chloroform was then evaporated at 40°C using a vacuum rotary evaporator. After evaporation, Tween 80 (400 mg) and linoleic acid (40 mg) were mixed then added to 100 mL of distilled water. The mixture was vigorously shaken for 10 min. An emulsion without β-carotene was prepared and used as blank. After the addition of 50 µL of MgOr and MgOrGANPs at several concentrations and 200 µL of β-carotene emulsions into the 96-well plate, the mixture was incubated in the dark at 50°C. The absorbance was recorded at 470 nm and 20 min intervals for a total of 100 min. The antioxidant activity of MgOr and MgOrGANPs was calculated using Eq. 2:

$$AA_0 = \left[1 - \frac{(A_0 - A_t)}{(A_{0c} - A_{tc})}\right] \times 100 \qquad \text{(Eq. 2)}$$

Where A_0, A_t, A_{0c}, and A_{tc} = absorbance of sample at t = 0 and t = 100 min; and absorbance of control at t = 0 and t = 100 min, respectively. Various concentrations of α-tocopherol were used as standards (Barros *et al.,* 2007a).

Antihypertensive assay using angiotensin converting enzyme (ACE)

In this analysis, the examination of *in vitro* ACE inhibition activity was performed to quantify the conversion of hippuryl-histidyl-leucine to hippuric acid in the presence of an inhibitor enzyme. Briefly, 100 µL of MgOr and MgOrGANPs were reacted with ACE (25 µL, pH 8.3), and the reaction was pre-incubated for 5 min at 37°C. To quantify the ACE inhibition activity, an addition of 10 µL of hippuryl-histidyl-leucine (3.5 Mm) to the assay mixture was then made followed by incubation for 30, 60, and 90 min. In order to stop the reaction, 50 µL of 3 mol/L HCI was added to the mixture. Ethyl acetate (1 mL) was added to extract the hippuric acid formed. Following evaporation at 120°C, the solvent was re-dissolved in deionised water. The quantification of hippuric acid was performed by measuring the absorbance at 228 nm (Hussein *et al.,* 2012). The reduction in absorbance recorded was proportional to the inhibition performed by the assayed inhibitor. The emulsion prepared without MgOr and MgOrGANPs served as control.

Cell culture and cytotoxicity assay

Four human cell lines were purchased from ATCC; human colon cancer (HT29), human liver cancer (HepG2), human breast cancer (MCF7), and normal breast cancer (MCF10a). Brielfy, 200 µL of a 1×10^4 cells/mL suspension were seeded into each well of the 96-well plate. The plates were then incubated for 24 h at 37°C with 5% CO_2 to ensure attachment at 70 - 80% confluency. Following incubation, the media was removed and the cells were treated with fresh media (200 µL) including MgOr and MgOrGANPs at various concentrations of (15.6 to 1,000 µg/mL). The plates were then incubated with the compounds for 72 h at 37°C with 5% CO_2. The cell viability was determined based on the MTT assay. After that, the media was aspirated of and the wells were washed three times using PBS to ensure that MgOr and MgOrGANPs were removed. Next, 200 µL of fresh media was added to each well and replaced with 200 µL of MTT-including culture

medium. Then, 20 µL of MTT solution (5 µg/mL) made to a total of 200 µL of culture medium was added to each well, mixed gently and incubated for 4 h at 37°C with 5% CO_2. The MTT+culture medium was replaced with 200 µL/well of DMSO in order to dissolve the formazan crystals formed. The absorbance was measured at 570 nm using microtiter plate. All experiments were performed in triplicate. The concentration of drug required to inhibit 50% of cell growth was calculated from the dose-response curves from each cell line and each compound (Al-Qubaisi *et al.,* 2013).

Statistical analysis

All experiments were done in triplicate. Statistical analysis was carried out using SPSS 16 for Windows. The differences among the treatment groups was evaluated using one-way analysis of variance (ANOVA). Differences at $p < 0.05$ were considered significant (Sodagar *et al.,* 2013).

Results

DPPH scavenging activity of MgOAGANPs

The DPPH assay is based on the reduction capacity of DDPH in the presence of antioxidant compounds by donating an electron or a hydrogen to the free radical. Both MgOr and MgOrGANPs showed an antioxidant activity at the concentrations of 50, 75, 100, 125, 150, and 200 µg/mL (Figure 1) with an IC_{50} of 38.21 and 61.65 (µg/mL) for MgOrGANPs and MgOr, respectively.

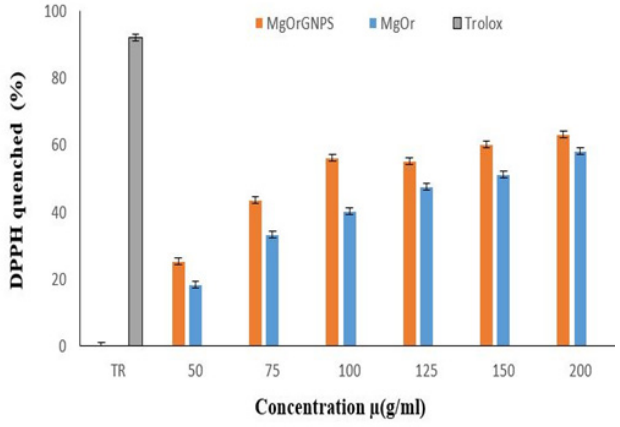

Figure 1. The DPPH scavenging activity at various concentrations of free MgOr and MgOrGANPs.

Nitric oxide (NO) radical scavenging activities of MgOrGANPs

Results showed that the cells treated with MgOr and MgOrGANPs exhibited a considerable inhibition of NO production as illustrated in Figure 2 with the hydroxyl radical scavenging activity of , MgOrGANPs appearing to be higher than that of MgOr.

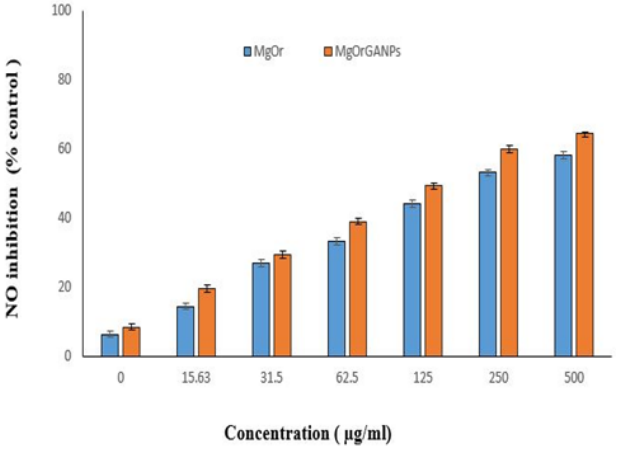

Figure 2. Nitric oxide radical scavenging activities of RAW 264.7 cell line treated with different concentrations of free MgOr and MgOrGANPs.

β-carotene bleaching assay (BCRA)

The β-carotene bleaching assay is usually used to evaluate the loss of yellow colour of β-carotene reacting with radicals in the presence of linoleic acid in the emulsion. In the present work, the antioxidant activities of MgOr and MgOrGANPs were investigated using the linoleic model system. The results confirmed the radical scavenging activity of MgOr and MgOrGANPs at various concentrations as shown in Figure 3.

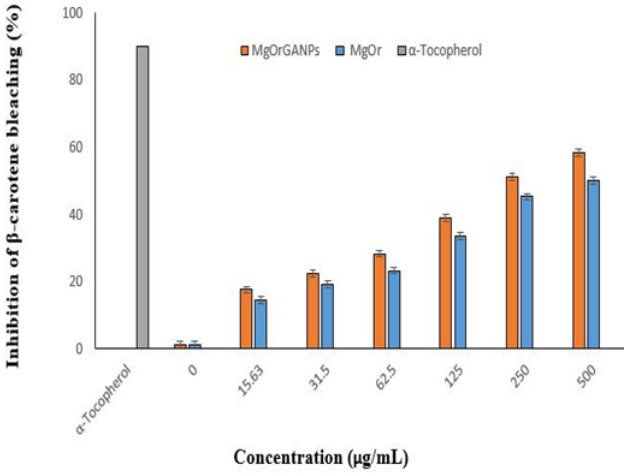

Figure 3. Inhibition activity (%) of free MgOr and MgOrGANPs at various concentrations by β-carotene bleaching assay.

Antihypertensive assay using angiotensin converting enzyme (ACE)

The *in vitro* antihypertensive activity of MgOr and MgOrGANPs were evaluated by converting hippuryl-histidyl-leucine to hippuric acid and measuring the ACE-inhibitory percentage based on simple absorbance measurements at 228 nm as shown in Figure 4.

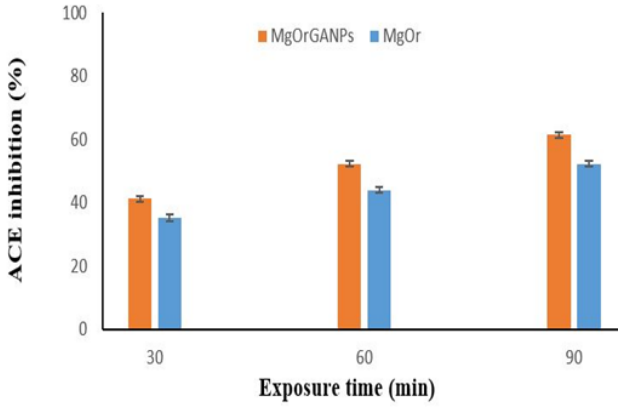

Figure 4. ACE inhibition (%) for free MgOr and MgOrGANPs at various concentrations after 30, 60, and 90 minutes exposure time.

Cytotoxicity assay

The growth inhibition and cytotoxicity of MgOr and MgOrGANPs towards human colon cancer (HT29), human liver cancer (HepG2), human breast cancer (MCF7), and normal breast cancer (MCF10a) cell lines were investigated using MTT assay. The cells were similarly treated with MgOr and MgOrGANPs at various concentrations of 1.56, 3.12, 6.25, 12.50, 25, 50, and 1000 µg/mL for 72 h. A substantial morphological changes appeared in HepG2, MCF7, and HT29 cell lines after the treatment of MgOr and MgOrGANPs as shown in Figure 5., HepG2 appeared to be more sensitive to MgOr and MgOrGANPs with an IC_{50} of 24.97 ± 0.1 and 12.83 ± 0.2, respectively, while MCF10a was the least sensitive with an IC_{50} of 270.74 ± 0.09 and 302.34 ± 0.15 for MgOr and MgOrGANPs, respectively. Additionally, when compared with MCF10a cells, the IC_{50} value of MgOrGANPs was about 20 times higher than that recorded in HT29 and MCF7 cells.

Discussion

In the present work, the NPs were successfully prepared by using GA polymer. The nano-encapsulation of MgOr improved its stability and antioxidant properties. The preparation of MgOrGANPs required an efficient stabiliser like GA to prevent aggregation and control growth. GA is commonly used as both stabilising and reducing agents. The purpose of using deionised water during the encapsulation of MgOr into GANPs was to improve the dispersion of particles using sonication in order to prepare NPs at the desired concentrations (Shi *et al.,* 2011).

The DPPH assay demonstrated the reaction between the free radical and antioxidants. Therefore, the electron became pairs and resulted in decreased absorption. This test was characterised by the reduction of DPPH with a colour change from purple to colourless or pale yellow. The decolourisation of DPPH was quantified based on the variation in the absorbance values (Ndhlala *et al.,* 2010). The DPPH

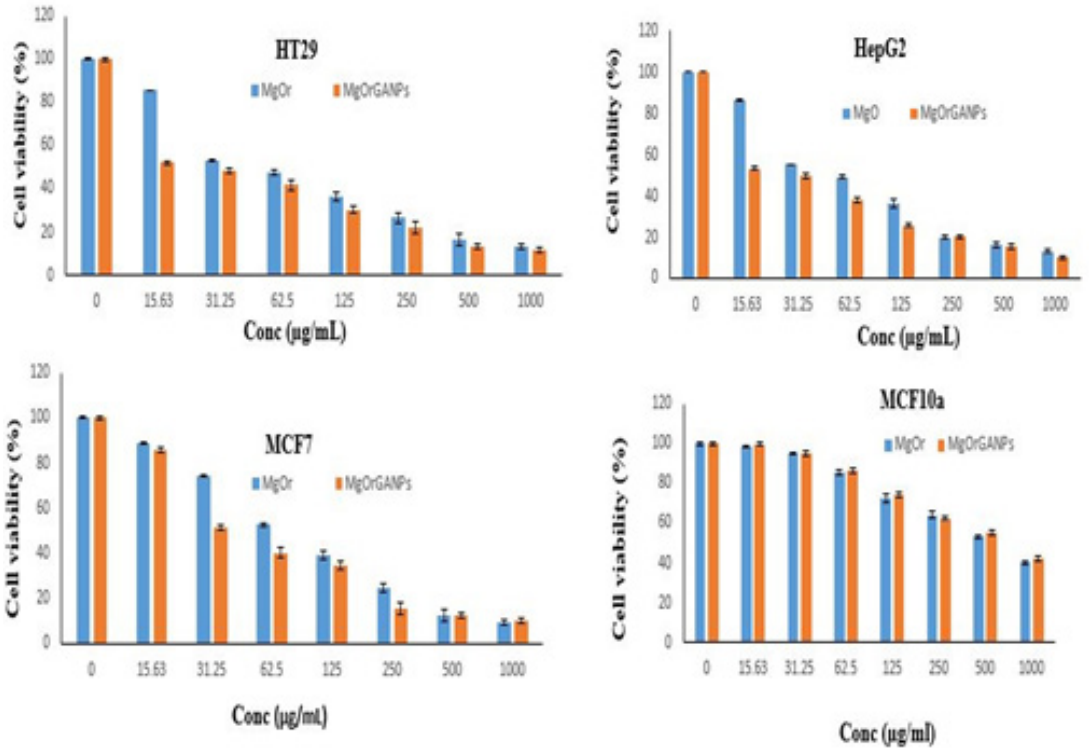

Figure 5. MgOr and MgOrGANPs effects on the viability of treated cells using MTT assay.

results showed strong radical scavenging activities of MgOr and MgOrGANPs.

The investigation on radical scavenging activities presented excellent correlation between MgOr and MgOrGANPs before and after encapsulation into the GA polymer. GA is a high molecular weight biopolymer with a variable mixture of glycoproteins and polysaccharides. Hence, GA, which acted as both coating and reducing agents, increased the percentage of oxidation inhibition of NPs. The reduced inhibition was attributed to the reduced particle surface area available for the scavenging of DPPH radicals in the presence of large clusters of samples. The rate of absorption recorded at 517 nm was influenced by the odd electron of DPPH during the coloration process, and it accepted an electron donated by the antioxidant compound. MgOrGANPs displayed higher active scavenging activity as compared to MgOr due to their capacity to scavenge the stable free radical (DPPH) that reacted with the hydroxyl groups of GA. The results indicated that the encapsulation of MgOr into GANPs improved its antioxidant properties. Antioxidant activities of GA has been established previously which confirms its pharmaceutical applications as a nanocarrier for tumour-specific treatment (Gamal el-din *et al.,* 2003).

The nitric oxide (NO) assay has major importance for several types of inflammatory processes. NO, as a gaseous free radical, was responsible for various beneficial biological effects (Dowding *et al.,* 2012). NO assay is focused on the interaction between oxygen and NO being generated spontaneously by sodium nitroprusside in aqueous solution at physiological pH. These interactions generated nitric ions that could be evaluated using Griess' reagent.

The pre-treatment of the cells with MgOr and MgOrGANPs at various concentrations inhibited the lipopolysaccharide (LPS) stimulation of NO accumulation. Furthermore, scavengers compete with oxygen in order to decrease the production of nitric ions. It has been shown that the production of NO is induced by bacterial LPS (Wang and Mazza, 2002).

The MgOrGANPs yielded effective inhibition of NO secretion at concentrations ranging between 62.5 and 500 µg/mL under a concentration-dependent manner. The significant radical scavenging activity at 500 µg/mL was related to the antioxidant activity of GA. The analysis revealed that MgOrGANPs yielded higher inhibitory activity than the pure MgOr with an IC_{50} values of 56.9 and 89.56 µg/mL, respectively ($p < 0.05$). This findings confirm that MgOr protects its antioxidant activity. However, the mechanism causing the downregulation of NO using MgOr and MgOrGANPs has not been clarified. The potent antioxidant activity of GA is linked to the protein fraction of GA that corresponded to 0.76% (w/w) of this coating material. In addition, the potent antioxidant activity of GA against NO might be related to the groups of amino acids tyrosine, methionine, and histidine. It has been reported that GA has antioxidant scavenging activities under specific *in vitro* conditions by affecting the scavenging activity of the resulted NO (Gamal el-din *et al.,* 2003). The biopolymer might be linked with the interaction between the encapsulated agent and NO due its properties as membranes semipermeable to oxygen while molecules with similar volume to oxygen can penetrate into the nanocarrier, and subsequently scavenged by MgOr.

In general, the antioxidant compounds are frequently used in the treatment of various diseases due to their scavenging capacities (Vivekananthan *et al.,* 2003). Antioxidant is also a potent inhibitor, which may explain its important effect in cancer treatments and reactive oxygen species (Ranneh *et al.,* 2016). Furthermore, various side effects caused by the excessive level of NO radical produced including toxic effects are observed in specific tissues and inflammatory conditions, such as ulcerative colitis, multiple sclerosis, and juvenile diabetes mellitus (Mikkelsen and Wardman, 2003).

β-carotene is a natural carotenoid deemed as a major precursor of vitamin A and has been established as a free radical scavenger with potential chain-breaking antioxidant activities. It is a powerful antioxidant, derived from plants and protects human body against the attack of free radicals (Albanes *et al.,* 1996; Yusuf *et al.,* 2012). In the biosystem, β-carotene tends to convert into retinol due its antioxidant properties and can also protect nervous system against neurodegenerative disorders (Obulesu *et al.,* 2011).

The principle of β-carotene assay is primarily based on linoleic acid oxidation, which results in a decrease of β-carotene's yellow colour. In the present work, the degradation of β-carotene was inhibited by the presence of MgOrGANPs as antioxidant. Interestingly, the values of β-carotene antioxidant activity were increased in a dose-dependent manner. The MgOrGANPs exhibited much higher levels of antioxidant activity than MgOr which can be related to the functional groups of GA used as nanocarrier. The data revealed a link between antioxidant effects of MgOr and MgOrGANPs, and its concentration as well as the decrease of β-carotene loss in samples.

The results mentioned a concentration-dependent inhibition of β-carotene, while the concentration of MgOrGANPs of 500 µg/mL and 58.4% of antioxidant activity ($p < 0.05$) appeared to be significantly higher than the other concentrations. These differences can be explained by the oxygen scavenging property of β-carotene that influenced the oxygen functionalities on MgOrGANPs. The amount and types of antioxidant influence the stability of β-carotene. A previous study revealed a slower degradation of β-carotene in the presence of GA with higher concentration of antioxidants. The main role of antioxidant is to protect biosystem against reactive oxygen species (ROS) such as hydrogen peroxides and hydroxyl radicals (Castro *et al.,* 2006). Thus, the therapeutic effect of NPs may be associated with its ability to scavenge free radicals.

On that account, the presence of GA in MgOrGANPs is protective against the attacks of lipoxygenase and free radicals BC5 as well as the targeting specificity of the nanocarrier based on the effect of asialoglycoprotein receptors. Moreover, GA has excellent potential for delivering bioactive agents as a selected and target-oriented delivery system which functions through receptor mediated endocytose (RME) (Banerjee and Chen, 2009). Hence, this suggests that GA improves the antioxidant activities of MgOr during the encapsulation process.

The present work was the first to perform an evaluation of ACE inhibitory activity of MgOr by loading it to a GA nanocarrier. The measurement of ACE-inhibitory percentage based on the absorbance measurements at 228 nm exhibited that the percentage inhibition of MgOr and MgOrGANPs was proportional to the decrease of absorbance recorded. Similarly, orotic acid is an anti-hypertensive biomolecule used as an intermediate in pyrimidine synthesis. ACE inhibitors are commonly prescribed to treat high blood pressure. The ACE inhibitory effects of MgOrGANPs was evaluated and measured as ACE-inhibitory percentage using the study samples. The effects of MgOr and MgOrGANPs on ACE are depicted in Figure 4. There was a slight increase in ACE of MgOrGANPs after 90 min as compared to the measurements at 30 and 60 min. After the incubation of 100 µL MgOrGANPs (5 µg/mL) with ACE, the results revealed that 41.26, 52.34, and 61.45% in ACE were recorded after 30, 60, and 90 min, respectively ($p < 0.05$). MgOrGANPs were demonstrated to have more potent ACE inhibition activity as compared to MgOr. This could be explained by the high amount of MgOr released from the GA NPs. Hence, it is suggested that GA could improve the antihypertensive capacity of MgOrGANPs. Nonetheless, further research is required to validate the findings. As a highly heterogeneous compound, GA is incorporated with approximately 2% of polypeptide, which is an active substance that improves the antihypertensive effect.

The role of GA NPs in biomedical applications can be related to their unique properties, particularly in drug delivery system which confirms their high stability and low toxicity (Castro *et al.,* 2006). *In vitro* cytotoxicity tests can be used to screen biocompounds before testing it *in vivo* (Kong *et al.,* 2014). As shown in Figure 5 , HT29, HepG2, and MCF7 cells responded differently against MgOrGANPs in exposure media. After 72 h, MCF10a showed the highest resistance to the NPs, and HepG2 appeared to be more sensitive to MgOrGANPs than MCF7 and HT29. The purpose of using MCF10a as a normal cell lines is to compare the cytotoxicity effect of MgOrGANPs towards MCF7 cell lines which can be considered as an important requirement in the case of formulated drugs. In previous study, biologically targeted NPs have been developed to identify differences between normal Chinese hamster ovary cells and MDA-MB231, MCF7, and BT-474 breast cancer cells. This is an important consideration in treating cancer cells as well as protecting normal cells (Al-Qubaisi *et al.,* 2013).

For HepG2, the lower percentage of cell viability was recorded at the concentration of 1,000 µg/mL of MgOrGANPs, while at the concentration of 15.63 µg/mL, 53.23% of cell viability was recorded. However, the concentration of 1,000 µg/mL of MgOr stimulated lower percentage of cell viability (13.3%). Furthermore, the concentration of 15.63 µg/mL showed 86.22% of viable cells as compared to the untreated cells ($p < 0.05$). In a previous study by Nishi *et al.* (2007), the mechanism of GA NPs targeted to the liver has been reported based on the interactions between asialoglycoprotein receptors (ASGRP), the function of receptor-mediated endocytosis (RME), and nanocarriers (Sarika *et al.,* 2015). The results mentioned that the anticancer activity of MgOr loaded into GA NPs was slightly higher than the free MgOr due to the specific properties of GA polymer. The IC_{50} values are shown in Table 1. Consequently, the identification of the asialoglycoprotein receptors (ASGRP) using galactose moiety on the surface of hepatocytes have increased the toxicity of MgOrGANPs. Additionally, GA conjugates have been also tested for controlled and sustained-release drug delivery system accordingly. MgOrGANPs have also exhibited cytotoxicity and improved tumour targeting against the HepG2 cells. This may be due to the targeting efficacy of the

Table 1. IC_{50} of MgOrGANPs, MgOr, 5-Fluorouracil, Doxorubicin, and Tamoxifen on HT29, MCF7, HepG2, and MCF10a cell lines following 72 h incubation.

Treatment	IC_{50} (µg/L)			
	HT29	MCF7	HepG2	MCF10a
MgOrGANPs	15.9 ± 1.2	15.24 ± 1.4	12.83 ± 1.1	270.74 ± 1.5
MgOr	29.4 ± 1.1	32.46 ± 1.3	24.97 ± 1.3	302.34 ± 1.4
5-Fluorouracil	1.064 ± 0.8	---	---	---
Doxorubicin	---	0.083 ± 0.78	---	---
Tamoxifen	---	---	1.028 ± 0.9	---

galactose groups of GA. GA NPs have also shown increased uptake caused by the malignant liver cancer cells (Sarika *et al.,* 2015).

Conclusion

The results confirmed the antioxidant and antihypertensive properties of MgOrGANPs. MgOrGANPs also showed potent antioxidant activity than MgOr due the effect of gum arabic polymer. The present work demonstrated that MgOrGANPs can be considered as a potential candidate compound for the evaluation of prevention and treatment of cancers.

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